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Studies on the Preparation, Immobilization, and Luminescence Properties of Zinc Oxide (ZnO)Quantum Dots

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by

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ABSTRACT

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Quantum dots are a part of very important our technological future because of their intriguing and useful properties. These properties are different in character from those of the corresponding bulk material. Quantum dots are inorganic artificial semiconductor nanocrystal whose electrons influence their physical and chemical properties. Zinc oxide quantum dots were synthesized through an ethanol based precipitation via colloidal synthesis method at various pH values. Various emission colors were obtained because the excited quantum dots of various sizes emitted specific wavelengths of light. The emission spectra indicated that the pH dependent quantum dots were successfully synthesized. Zinc oxide quantum dots were also encapsulated and the luminescence properties examined. The quantum dots that were immobilized in polyisoprene (PI) through chemiluminescence (CL) analyses were found to be stable and were capable of continuing their luminescence properties with extended uses and long- term storage. Linear calibration curves were acquired for concentration of 8.75×10^{-5} M H_2O_2 in TCPO-CL.

DEDICATION

To my loving son Tekin, my mom Nesime hanim, my dad A. Remzi bey

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CHAPTER 1

INTRODUCTION

Nanotechnology is the study of matter at atomic and molecular scale and generally deals with structures size between 1 to100 nm in at least one dimension by the quantum mechanical effect. At such small size scales, quantum mechanical effects give rise to novel materials behavior compared to their corresponding bulk materials (1). Nanotechnology has an enormous scientific and practical future in many emerging fields. Materials on the nano scale often exhibit very different physical, chemical, and biological properties than their normal size counterparts.

In recent years, a tremendous amount of research effort has been made towards synthesis, properties, manipulation, and characterization of the nano scale materials. It is receiving increasing attention of research laboratories, fabrication, and manufacturing plants that are developing novel nano sized materials in many possible applications such as medicine (drug development), security, communication, transportation, energy, electronics, biomaterials, engineering, manufacturing, energy production, information, and others.

History of Nanoparticles

The concept of nanotechnology was first introduced by the well-known physicist Richard Feynman (2). Feynman described the ability to manipulate individual atoms or molecules with new experimental techniques, instrumentation, and process to modify matter and visualized them with the atomic precision could be extremely advantageous. The bulk materials have constant physical properties regardless of their size, and working with them was very challenging. Thus, Feynman had a great interest in working materials on the nano scale with size dependent properties. Later, an engineer named Eric Drexler studied these concepts of nanotechnology in

greater depth (3). All these research efforts led to the discovery of fullerenes (4), semiconductor nanocrystal (5), and other nanoparticles (6-11).

Fullerenes

The chemical and physical properties of fullerenes have been a great topic in the field of nano scale research and development. The investigation of nanoparticles began with the discovery of fullerenes, any molecule composed of carbon atoms that is neither graphite nor diamond. In the 1980s, fullerenes were discovered by Richard Smalley and Robert Curl, Jr. of Rice University in Houston, Texas (4) and Harold Kroto of Sussex University in the United Kingdom (12). Unlike diamond or graphite, fullerenes consist of 60, 70, or even more carbon atoms. Fullerenes actually can occur naturally (13). Now many techniques are available synthesizing them in greater amount. Fullerenes are used in biological and medical applications (4, 14). For instance, they can be used as antioxidants while react with free radicals that cause to damage normal cells. Major pharmaceutical companies are exploring the use of fullerenes in controlling the neurological damage of such diseases as Alzheimer's disease (14).

Semiconductor Nanoparticles

Nanoparticles are particles with diameters of 100 nm or smaller (15). Materials on the nano scale and semiconductor nanoparticles are of great interest because of their size dependent optical, catalytic, and electrical properties (16). The differences in property between the nanoparticles and bulk materials have stimulated many scientists to investigate their size dependent properties. Nanoparticles are a bridge between atomic or molecular structure and bulk materials. They are aggregates of multiple atoms or molecules so they have a larger size than an individual atom or molecule. Due to the small size of particles, they have a large surface area to

volume ratio. With the decreasing particle size, bulk properties are lost, electronic states becomes discrete, and the surface of the atoms becomes larger (17).

The properties of semiconductor nanocrystals depend on the particle size as well as shapes, structure, and surface state of the nanoparticles (18). When a particle gets very small, it exhibits quantum mechanical behavior. This is typically because nanoparticles have greater surface areas which cause them to be more reactive to certain other molecules. Larger surface area of the nanoparticles causes a lot of interactions between the mixed materials.

Nanoparticles' unique optical and electronic properties are due to the quantum confinement effect. Quantum confinement is the change of optical and electronic properties when the material sample has size 10 nanometers or less. The origin of quantum confinement is called zero dimensional nanocrystallites such as that in the quantum dots. As the size of the nanostructure decreases, the band gap size increases. The surface of the material becomes more significant because properties of nano scaled materials change as their size approaches the nano scale. The bulk material surface is insignificant in relation to the number of atoms in the bulk of the material. Suspension of nanoparticles is possible when the interaction of the particle surface with the solvent is strong enough to overcome density differences.

Nanoparticles are synthesized for desired applications. These applications are based upon the dispersion medium that contains the nanoparticles, the dispersion state, and the miscellaneous surface modifications involved (15). To improve stability, nanoparticles are either immobilized or undergo surface modification. With today's technology and sophisticated instrumentations, characterization and manipulation of nanomaterials are possible such as instruments transmission electron microscopy (TEM), scanning-electron microscopy (SEM),

scanning-probe microscopy (SPM), fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), ultraviolet-visible spectroscopy (UV-Vis).

Examples of Nanoparticles

As more research was conducted, different types of semiconductor nanoparticles were discovered. Here are some of these nanoparticles.

Gold nanoparticles have been synthesized and used in the diagnosis of genetic diseases and mutations (8). Because of their uniqueness in optical and electronic properties, gold nanoparticles are being investigated as carriers for drugs (19) and can be used to target tumors in vivo (20). The application and properties of colloidal gold nanoparticles depend on the shape of the nanoparticles. Rod like particles have both transverse and longitudinal absorption peak, and anisotropy of the shape affects their self-assembly (21).

Recent research has also introduced silver nanoparticles into the medical field. Silver nanoparticles have been synthesized and used in fighting against bacteria, viruses, and fungi (7). Improved silver nanoparticles have been developed to prevent infections. They destroy specific enzymes and suffocate the bacteria, virus, and fungi so that they do not infect surrounding tissues. They have been added to surfaces of stainless surgical steel blades and needles for faster wound healing in medical applications (7). Methods for synthesizing silver nanoparticles include thermal decomposition (22), laser ablation (23), microwave irradiation (24), and sono-chemical synthesis (25).

Zinc oxide nanoparticles are versatile semiconductor inorganic materials. Zinc oxide nanoparticle has attracted attention because of commercial demand for optoelectronic devices. It is oxidized material that belongs to the quartzite structure. It has higher absorption efficiency at

the small particle sizes. This semiconductor has several favorable properties, including good transparency, high electron mobility, wide band-gap, and strong room-temperature luminescence (26). ZnO nanoparticles allow extensive applications in many fields ranging from bio-imaging to optoelectronics.

Toxicology of Nanoparticles

In general, there a great deal of ongoing research on the toxicology of nanoparticles. The novel properties of nanoparticles mean they could find uses in a variety of applications as diverse as solar power to treatment of cancers. A recent study at the effects of ZnO nanoparticles on human immune cells has found varying levels of susceptibility to cytotoxicity (27). Fear over the potential dangers of nanoparticles had led to increasing calls for their tests and regulations. In past number of years several studies had indicated that exposure to specific nanomaterials can lead to adverse effects in humans and animals (28). Researchers working with nanoparticle toxicology had identified molecular mechanisms that may cause dangerous reaction when some nanoparticles come into contact with living cells. It was shown that some nanoparticles could give rise to reactive forms of oxygen that damage living cells. At the low concentrations of these molecules, cells can defend themselves by producing antioxidants. As concentrations of these reactive forms increase, however, cells become inflamed or eventually die. Because there is no authority to regulate nanotechnology based on the products, there are many products that could possibly be dangerous to humans.

Quantum Dots

Quantum dots are a part of our very important technological future because they possess intriguing and useful properties. These properties are different in character from those of the corresponding bulk material. Quantum dots are inorganic semiconductor nanocrystals whose

electrons highly influence their physical and chemical properties (29). They are synthesized from II-VI small band gap semiconductors (30-32). Although they are composed of hundreds and even thousands of atoms, they behave like a single atom (33, 34). The quantum dots have the ability to emit light when an electron absorbs radiation from an external energy source such as heat, electricity, or light. Subsequently, the electron jumps to an excited state in the conduction band leaving a positive charged hole in the ground state orbital. They have tunable band gaps that allow for the wavelength of the light they release to be by controlled changing the particle size. In addition, their surface is composed of non-polar coordinating ligand, thus quantum dots can be attached to a variety of molecules. This allows the quantum dots to be dispersed or dissolved in many solvents.

Generally, the smaller the size of the nanoparticles the larger the band gap, hence the greater the difference is in the energy between the highest valence band and the lowest conduction band becomes (35). Therefore, more energy is needed to excite the smaller quantum dot, so more energy is released when the electron returns to its ground state. Traditional semiconductors have optical and electronic limitations that are difficult to overcome. Also, their emission frequencies cannot be easily manipulated because the band gap energies are not easily changed**.** Regular semiconductor dye has an absorption spectrum that is narrow, not bell shaped, and cannot easily be tuned. Organic dye has a luminescence that has a broad spectral width that limits effectiveness to a small number of colors. The band gap of quantum dots changes with their size by the addition or subtraction of just a few atoms. The most apparent property is the emission of photons under the excitation radiation that is visible to the naked human eye as light. The wavelength of photons emitted depends not on the material from which the quantum dot is made but the size of the particles.

Properties of Quantum Dot

Size Properties. Quantum dots have a high surface area per unit volume; therefore, small quantum dots are not very stable. They have a tendency to grow (5). Because the band gap of the quantum dots is easily changed with their size, the band gap energy also affects the size of quantum dots. Band gaps are spaces in between energy levels and the forbidden zones for electrons. The same material of quantum dots, but with different sizes, can emit different colors of light due to quantum confinement effect. The band gap energy that determines the energy of the fluorescent light is inversely proportional to the size of the quantum dot. Larger quantum dots have more energy levels so they are also closely spaced in which the electron hole pair can be trapped. The electron hole pairs in larger dots live longer causing larger dots to show a longer lifetime. As a result, when the electron returns to its ground state, longer wavelengths of light are emitted (35).

Reducing the size of regular semiconductor materials to nanometer scale changes its physical properties in a fundamental way. For instance, their sizes produce optical effects (26). Smaller quantum dots emit shorter wavelengths, toward the blue end of the visible spectrum, while larger quantum dots emit longer wavelengths of light, toward the red end of the visible spectrum (36, 37). In addition, free quantum dots can be bound to molecules that can influence magnetic behavior, sintering, and melting temperatures of the materials.

Surface Properties. The unusual and often unexpected properties of nanoparticles are largely due to the large surface area of the semiconductor material. Size and shape monodispersity is very important to carefully reveal the dependence of the material's properties on size or shape and therefore requires synthetic routes to prepare monodispersed nanocrystals (38). At elevated temperatures, the high surface area to volume ratio of quantum dots contributes

a huge driving force for diffusion. Nanoparticles have been found to show some extra properties to change day to day products. Quantum dots enhance catalytic activity due to their large surface area per unit volume. Examples of strategies used to make quantum dots more biocompatible include coating with a silica layer (39) and encapsulating the hydrophobic quantum dots in lipid micelles (40). Recently, encapsulation of quantum dots into polymer colloids is widely used techniques because it is robust, facile, and inexpensive.

Applications of Quantum Dots

To ability to tune the size of quantum dots offers great advantageous in many applications. For example, larger quantum dots have a larger spectral shift towards red compared to smaller quantum dots. The new generations of quantum dots have far reaching potential for the study of intracellular processes at the single molecule level, high resolution cellular imaging, long-term *in vivo* observation of cell trafficking, tumor targeting, and diagnostic (35).

Biological Applications. The most successful application of quantum dots is use as biological tags that involve modification the quantum dot surface specifically for imaging biological targets. In modern biological analysis, quantum dots are replacing organic dyes. However, with each passing year, more demands are required of these dyes, and the traditional dyes are often unable to meet the expectations (41). Researchers have studied and compared the optical properties of quantum dots to the organic dyes. Quantum dots have being found to be superior to traditional organic dyes on several counts. Similar to quantum dots, organic dyes absorb radiation to excite an electron and emit energy when the electron returns to its ground state. One of the most obvious properties of the quantum dots are its brightness.

Photobleaching can occur with most organic dyes after they have been exposed to light for long period of time. It has been estimated that quantum dots are 20 times brighter and 100 times more stable than traditional fluorescent reporters (41). The color produced by the quantum dots last for weeks or longer, which provides more time for observation and characterizations (42, 43). Finally, organic dyes fluoresce at distinct laser excitation wavelengths, and one color is observed at a time. On the other hand, quantum dots emit light of different wavelengths when excited.

Medical Applications. Quantum dots are used in cancer research (33). Nanotechnology is opening up new opportunities in implantable delivery systems, which are often preferable to the use of inject able drugs. The side effects and drug consumption can be reduced significantly by depositing the active agent or higher dose that is needed at the specific site. This highly selective approach reduces cost and more importantly human suffering. Targeted or personalized medicine reduces drug consumption and treatment expenses resulting in an overall benefit by reducing the costs to the public health system. Researchers have conjugated quantum dots to antibodies to recognize cancer cell (33). Their small size combines with infinite variations of function allow them to interact with tumor cells in complex ways.

Technological Applications. One of the fastest moving and most exciting aspects of nanotechnology is the use of quantum dots in technology. Quantum computation is one of the most promising candidates for use in solid state quantum dot technology (35). Flow of electrons through the quantum dot can be controlled and precise measurements can be made. They are incorporated in more efficient integrated circuit chips and smaller, more powerful transistors for computers (44, 45). With quantum dots, information storage is brought down to the molecular level. Because there is no current of electrons required for transmitting the signal, problem of

removing heat in traditional semiconductors is avoided. Furthermore, the information processing by quantum dots occur at discrete states of single electrons. This allows the possibility of constructing ultrahigh density memory devices. Because of their tiny size, nanoparticles are inherently poised to aid in the production of high performance delicate electronics; they may provide not only materials with a high rate of conductivity but also sleeker parts for small consumer electronics like cell phones (46). Nanoparticles electronics can create digital displays that are brighter in color, electricity-efficient, and less expensive to produce. In addition, nanoparticles could provide improved wear and tear resistance for almost any mechanical device (46).

CHAPTER 2

PROPERTIES OF ZnO QUANTUM DOTS AND LUMINESCENCE TECHNIQUES

ZnO Quantum Dots

Research on the zinc oxide (ZnO) quantum dots is expanding tremendously due to their numerous attractive properties. As mentioned in the previous chapter, the properties of ZnO quantum dots are sensitive not only to the dimensions but also to the shape and other related complexities such as the surface effects and size variation. The properties of ZnO quantum dots contain crucial information regarding of many parameters that is essential to better understanding the quantum dots devices and applications.

Zinc and oxygen belong to the 12th and 16th group of the periodic table. ZnO is known as II-VI binary semiconductor compound. It has a high direct band gap of 3.37 eV and a large excitonic binding energy of 60 meV compared to other wide band material that allows electron transition even at room temperature (47). In addition, ZnO nanoparticles have an excellent UV absorption efficiency (48). Some other positive attributes include the long-term non-negative environmentally impact, bio-compatibility, non-toxicity, and low cost. For instance, ZnO nanocrystals usually are suitable for *in vivo* bio imaging because of non-toxicity effects.

The Zn based quantum dots provide physical blocks from harmful UVA and UVB rays (6). It is protecting the DNA or skin from photo damage by exposure to ultraviolet radiation. Applications making use of this property include UV-protective coatings for wood furniture and textiles and polymer-composite products to prevent degradation against harmful UV rays (48).

ZnO quantum dots are nearly insoluble in water and appear as a white crystalline powder. This powder is widely used as an additive in many materials. Commercially most ZnO nanocrystal is produced by the colloidal synthesis method. The vast majority of these

commercial applications use ZnO in its polycrystalline (powder) form, which is currently produced at a level of 100,000 tons per year (49). When kept in the dark, the quantum dots remain stable for days and even months (50).

A variety of chemical and physical methods are used to synthesize the ZnO quantum dots. Despite the development process of many nanoparticles, chemically pure, crystalline, and nano sized ZnO quantum dots can be easily synthesized. ZnO nanoparticle is almost 500 times larger than that of bulk ZnO (51). Therefore, applications of ZnO quantum dots can be improved simply by changing the particle size.

Synthetic Procedures of Quantum Dots

In order to take advantage of the quantum dots, knowledge of how quantum dot synthesis and growth can be controlled is required. The most popular synthetic procedures are vapor deposition, molecular self-assembly, mechanical size reduction, and colloidal synthesis (13). The method chosen for the synthesis of quantum dots is very important because the properties of the quantum dots change with different synthetic methods. For instance, a colloidal crystal is an ordered array of particles with diameters ranging from tens of nanometers to micrometers (52).

Colloidal Synthesis

The colloidal semiconductor nanoparticles are produced from precursor compounds. Colloidal synthesis is the most popular and most efficient method to produce quantum dots (50). This method of synthesis is based on three component systems that include the organic surfactants, solvents, and precursors. Precursors are chemically transformed into monomers by applying heat to them. Once the monomers reach a high enough super-saturation level, the nanocrystal growth starts with a nucleation process (35). During the nanocrystal growth process, pH is one of the critical factors in determining optimal (optoelectronic) conditions. Colloidal

synthesis is simple and can be used to synthesize large quantities of high quality nanoparticles at minimal cost (53- 55). Quantum dots size and shape are controlled easily as a result of growth. The smaller particles grow faster than larger ones resulting in focusing of the size distribution to yield nearly mono-disperse particles (35).

Vapor Deposition Synthesis

Vapor deposition synthetic method describes a variety of methods to deposit thin film by the condensation of a vaporized form of the material onto various surfaces (56). This method is used to produce high purity metallic or metal-oxide nanocrystals and often used in the semiconductor industry. The main advantage of this method is the low contamination level. Particle size is controlled by the variation of some the parameters such as pH, temperature, concentration, evaporation rate, and environment effect. The vapor deposition method is mainly used for coating and immobilizing quantum dots (56). The coating method involves purely physical processes such as high temperature vacuum evaporation or plasma sputter bombardment (56).

Molecular Self-Assembly Synthesis

In nanotechnology, molecular-self-assembly is a process in which quantum dots are synthesized from molecules without influence from an outside source. Molecular self-assembly is the "spontaneous" association of molecules under equilibrium conditions into stable, structurally well-defined aggregates joined by non-covalent bonds (57). Using this process, desired final structure is synthesized in the shape and functional groups of the molecules. Biological systems use this technique to assemble various molecules. The main advantage of this method is to construct nanostructure materials that eventually degrade back into individual molecules. Size and shape of nanoparticles can be controlled easily with this method.

Molecular self-assembly synthesis method produces a new class of semiconductor product at molecular level. Highly toxic surfactants are used during the process giving rise to environmental concern. The application of this method is quite simple, versatile, and provides scientists with new opportunities in the study nanoparticles. Chemical reactions construct atoms to assemble into larger structures will have an important role in the technology of the future.

Luminescence Techniques

Luminescence is emission of light by molecules or atoms. During the luminescence process, energy source excites an electron from atom or molecule of lower energy state into a higher excited energy state. When the electron returns to the ground energy state in the form of light is emitted. Fluorescence and chemiluminescence are the most common, powerful, and widely used luminescent techniques in the analytical chemistry. The widespread applications are due the significant advantages of selectivity and sensitivity of the luminescence techniques.

Fluorescence is the emission of light by a substance that absorbs external radiation sources such as heat, electricity, or light. Fluorescence technique is simple, sensitive, and selective. This technique also has a wide linear dynamic range that is advantageous quantitative analyses.

Chemiluminescence is the emission of light by release of energy from a chemical reaction instead of an external radiation source. Molecule loses excitation energy by emitting the light that can be in the ultraviolet, visible, or infrared regions. However, those emitting visible light are the most common (58). In order to obtain the highest sensitivity; chemiluminescent reactions must be as efficient as possible in generating photons of light. The attractiveness of this technique in nanotechnology as an analytical tool is the simplicity of detection method.

Principle of Fluorescence

Fluorescence spectroscopy is one of most popular analytical techniques for the characterizing of quantum dots in the wider linear dynamic range. The best method to understand the luminescence process is via a Jablonski diagram shown below in Figure 1 (59).

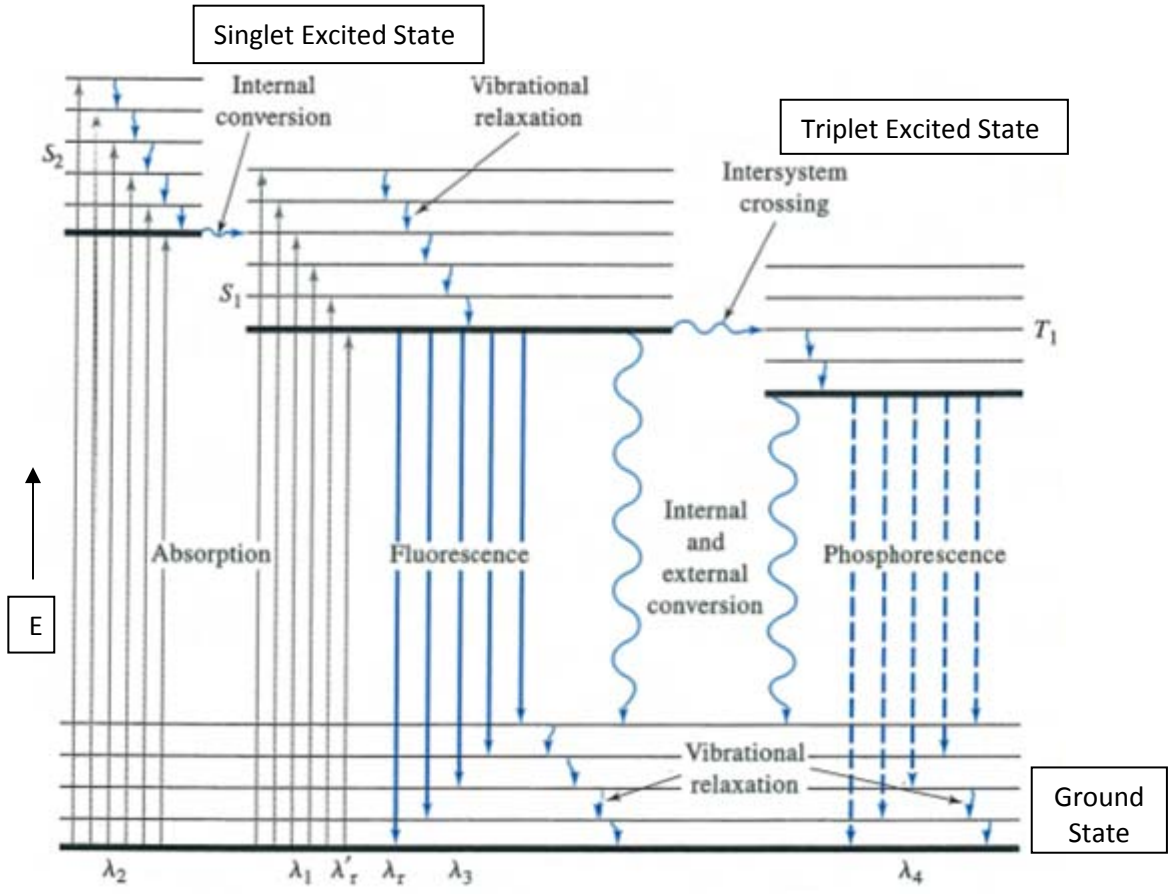

Figure 1. A Jablonski diagram for a fluorophore molecule adapted from (59)

The electronic ground state of a fluorescent molecule is represented by the horizontal lines, which are labeled in S_0, and is usually in the singlet state. The horizontal lines far above the ground state represent the second electronic singlet state (S_2), the first electronic singlet state (S_1), and the first electronic triplet state (T_1), respectively. The Jablonski diagram describes the

absorption and the emission of light by how the electrons in a fluorescent molecule are excited from lower ground state to higher excited states.

Fluorescent molecule usually exists in the ground state at room temperature. When it absorbs energy of a giving wavelength, an electron of the molecule is promoted to a higher excited state, whether S_1 or S_2. This takes place rapidly within 10^{-15} s. Stability of the electron in the excited state decreases due to excess of energy. This excess energy is dissipated via several pathways as the electron returns to ground state. The most common way to release this excess energy is simply by increasing the molecular vibrations. The vibrational relaxation takes place as energy is released by collisions with surrounding solvent molecules.

Radiative transition occurs between two states of fluorescent molecule. The first process is the absorption followed by the emission of light. Radiative deactivation of the electronically excited state occurs as an electronic transition between the electronic states other than the ground singlet. Once a molecule reaches the lowest vibrational level of the lowest electronic state, it can do several things, one of which is to return to the ground state by emission of a photon. This process is known as fluorescence. The average lifetime of fluorescence is 10^{-10} to 10^{-5} s. In most cases, the emitted light has a longer wavelength lower energy than the absorbed radiation.

Triplet state is in lower electronic energy than the excited singlet states. When the electron relaxes to its ground vibrational state (S_0) from the triplet state, the emission of radiation is known as phosphorescence (59). The frequencies of exciting and emitted light are dependent upon the particular molecules or atoms.

Radiationless transition is a process that occurs between two states of fluorescent molecule without emission of photon. The excited molecules relax from the lowest vibrational level of a higher electronic state (S_2) to a higher vibrational level of a lower electronic state (S_1),

via a process of internal conversion (IC). Potential energies of the two excited states remain equal, thus no energy is lost as the crossover occurs. Molecular spin state for internal conversion remains the same. In contrast, the spin of an excited electron be reversed, leaving the molecule in an excited triplet state; this process known as intersystem crossing and it competes with fluorescence (59). The intersystem crossing (ISC) is less probable because it involves a change spin state. In addition, the intersystem crossing is a forbidden transition due to quantum mechanical reasons.

Triplet state is in lower electronic energy than the excited singlet states. When the electron relaxes to its ground vibrational state (S_0) from the triplet state, the emission of radiation is known as phosphorescence (59).

Principle of Chemiluminescence

Chemiluminescence takes place by the generation of light when energy from a chemical reaction between the reagent and the analyte generates specie in the excited state. The intensity of light emission depends on the rate of the chemical reaction (58). Luminol chemiluminescence and peroxyoxalate chemilumunescence are most common chemiluminescence techniques that are applied in analytical chemistry applications. Luminol is widely used as a chemiluminescent reagent when combined with an oxidizing agent. Luminol chemiluminescence reaction is illustrated in the following equations:

$$A + B \rightarrow C^* + D \qquad [1]$$

$$C^* \rightarrow C + h\nu \qquad [2]$$

where A is a luminol reactant and B is an oxidant. C and D represent the products, and C* represents an excited specie of product C. The energy of emitted light is of frequency υ, and h is the Planck constant.

The bis(2,4,6-trichlorophenyl) oxalate (TCPO) chemiluminescence can also occur when an electronically excited species transfers its energy to another species that luminesces and is shown by the following equations.

$$A + B \rightarrow C^* + F \quad [3]$$

$$C^* + F \rightarrow F^* + C \quad [4]$$

$$F^* \rightarrow F + h\nu \quad [5]$$

where F is the fluorophore that the accepts transferred energy from the excited species C* (60).

Chemiluminescence reactions consist of a chemical reaction and luminescence process. Therefore, the number of reactions that result in significant chemiluminescence emission is comparatively small. In order for a chemiluminescent reaction to occur, the most important requirement is that there must be sufficient energy available for the formation of an excited state. The second requirement is that the excited state must be capable of losing this energy by the emission of visible photon or transferring its energy to another molecule that is luminescent. As a result, products of chemiluminescence reactions generally include one specie that is highly fluorescent. The intensity of chemiluminescence is based upon the chemiluminescence efficiency and number of reacting molecule, which is shown by the following equations:

$$I_{CL}=\varphi_{CL}\,(d[C]/dt \quad [6]$$

$$I_{CL}=\varphi_{EX}\,\varphi_{EM}\,(d[C]/dt) \quad [7]$$

where I_{CL} represents the intensity of radiant (photons per unit time), φ_{CL} is the chemiluminescence quantum yield (photons per reacting molecule), (d[C] / dt) stand for the rate of the chemical reaction, and φ_{EX} and φ_{EM} are the excitation and emission quantum yields. The chemiluminescence intensity decreases as the reagent is consumed over time (59).

Chemiluminescence measurement requirements are quite simple. First thing is to bring all reactants and then measure the intensity of the light emission. Instrumentation requires only a single light detector such as a photomultiplier tube (PMT) and a reaction vessel. The instrumentation does not require an external excitation source. Therefore, chemiluminescence measurements or detections are not limited by excitation light scattering. Reagents and solvents used for this process must be of the highest purity. Chemiluminescence takes place is popular many among spectroscopic techniques because of its high selectivity and sensitivity. The detection limits are dependent upon reagent purity and lie between parts per billion and parts per million ranges (59).

Peroxyoxalate Chemiluminescence

Rauhut and co-workers synthesized and characterized the chemiluminescent properties of peroxyoxalates (59). The initial excited state does not emit light, instead it produces the highly energetic intermediate compounds. The reaction between other oxalates, including oxalic anhydrides and substituted phenyloxalates, and hydrogen peroxide were also researched (61, 62). The mechanism for the peroxyoxalate chemiluminescence reactions are shown in Figure 2 below (60).

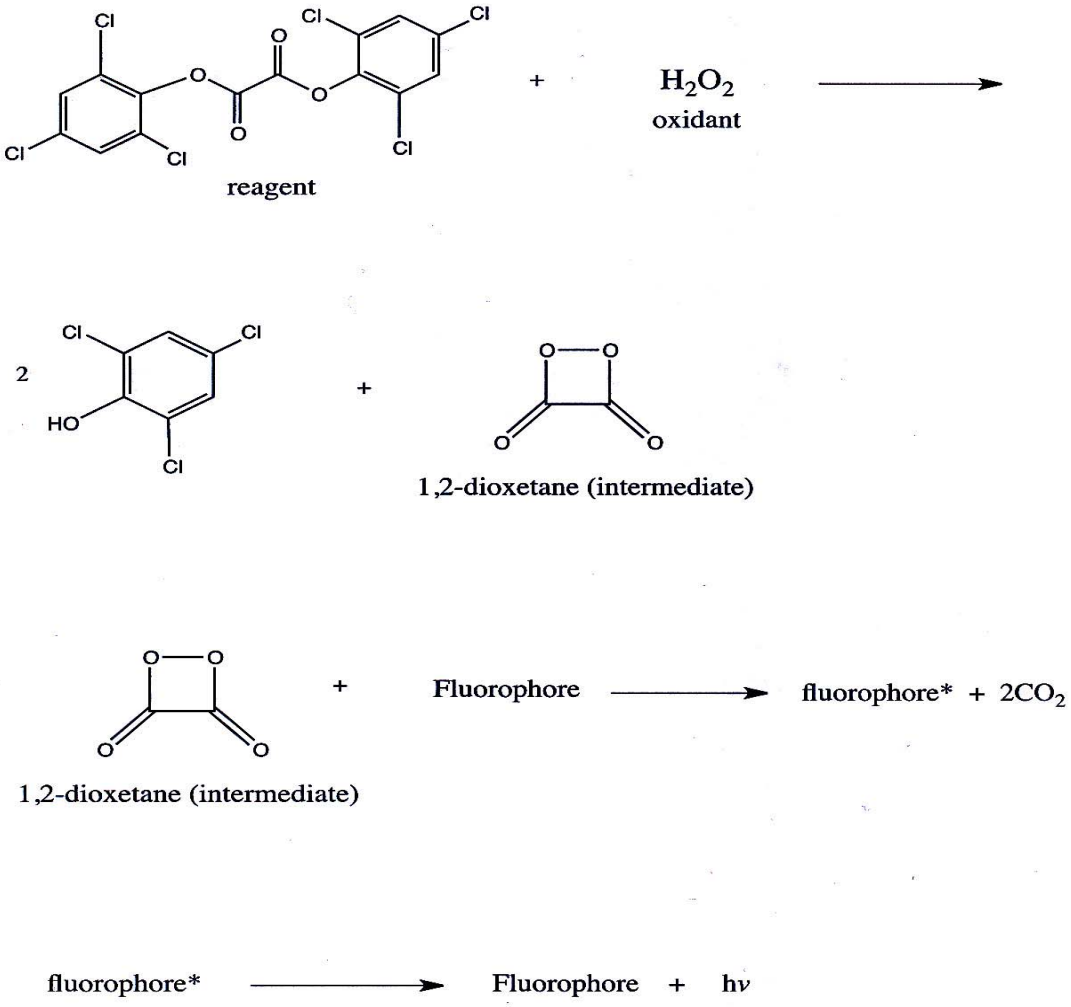

Figure 2. Chemical mechanisms of peroxyoxalate chemiluminescence reaction.

According to the given reactions above, in the presence of peroxyoxalates, of which bis(2,4,6-trichlorophenyl)oxalate (TCPO) used as a reagent and reacting with the oxidant (H_2O_2) to produce 1,4-dioxetanedione intermediate. This intermediate provides the chemical energy required for the excitation process. The fluorophore emits light as the excited species returns to its ground vibrational state (60).

Peroxyoxalate chemiluminescence is simple and inexpensive process with a high degree of selectivity compared to other chemiluminescence analytical methods. This type of chemiluminescence method also has fast analysis times and good sensitivity. In addition, peroxyoxalate chemiluminescence can be applied to various types of analysis because different fluorophores can be used (63, 64). Peroxyoxalate chemiluminescence has been used in analyzing immobilized amino aromatics as fluorophores (65). It has also been used to analyze glucose in urine (64). Peroxyoxalate chemiluminescence detection offers several advantages in chemiluminescent analyses. First of all, the TCPO peroxyoxalates can be synthesized quite easily (58).

Motivations

Based on the information and discussion presented in Chapter I and Chapter II, ZnO quantum dots can be synthesized via a colloidal synthesis method by adjustment of the pH of the precipitation solution. It is a simple procedure, and large quantities of high-quality quantum dots can be produced at minimal cost (53- 55). This procedure is now an effective way of synthesizing ZnO quantum dots. Also, ZnO quantum dots with various emission colors can be tuned through this colloidal synthesis method. In addition, fluorescent ZnO quantum dots can be encapsulated using an emulsification/solvent evaporation method to overcome the difficulty that is water solubility.

In this research project, an attempt was made to try the repeated synthetic method to obtain the ZnO quantum dots (QDs) needed for immobilization and use as a fluorophore in chemiluminescence (CL) analysis. We carried out experiments to investigate the following:

- Investigate the synthesis of ZnO QDs through an ethanol-based precipitation method at the various pH values.
- Investigate the procedures to immobilize the ZnO QDs into PI polymer by emulsification/solvent evaporation.
- Investigate the optimum concentrations of imidazole and TCPO for CL measurements of the PI immobilized QDs.
- Investigate the figures of merit of the proposed procedure, the precision, linear dynamic range and stability of the PI immobilized QDs.
- Investigate the luminescence properties of PI-QDs nanocomposite particles via fluorescence and chemiluminescence.

CHAPTER 3

EXPERIMENTAL PROCEDURE

In order to validate the applicability of the proposed research of the ZnO quantum dots, a number of experiments were performed to determine the accuracy, linearity, and precision of the luminescence signal. In this chapter, the experimental procedures to synthesize, immobilize, and establish the validity of the fluorescence and chemiluminescence properties of ZnO quantum dots are indicated.

The ZnO quantum dots were synthesized by using an ethanol based precipitation method via adjustment of the concentration of LiOH (66). The flourescent quantum dots were encapsulated into polyisoprene particles through an emulsification/solvent evaporation method (67).

Reagents Used

ACS certified commercial sources reagents were used. No additional purification procedures were required.

1. Cyclohexane, 95 % ethanol, 30 % hydrogen peroxide, lithium hydroxide, sodium hydroxide, benzene, lauric acid, and chloroform purchased from Fisher Scientific (Pittsburgh, PA).
2. Zinc acetate purchased from Mallinckrodt Baker (Phillipsburg, NJ).
3. 99 % Lauric acid, acetonitrile, and 90 % 1- octadecene purchased from Acros Organic Company (Morris Plains, New Jersey).
4. 99 % Imidazole, 98 % oxalyl chloride, 99 % triethylamine, and polyisoprene purchased from Aldrich Chemical Company (Milwaukee, WI).

5. 98 % 2, 4, 6 - Trichlorophenol purchased from Alfa Aesar Company (Pittsburgh, PA).
6. Deionized water acquired from US Filter Company (Pittsburgh, PA)

Preparation of Stock Solutions

Zinc Oxide Precursor Solution

Approximately 26 mg of ZnO nanoparticles and 2.0 mL of absolute ethanol were placed in a 25-mL flask. The solution was stored at room temperature.

Imidazole Solution

Imidazole solution (100 mg/mL) was prepared by dissolving 5.0 g of imidazole in 50 mL of deionized water. The solution was kept in the refrigerator.

Hyrogen Peroxide Solution

The hydrogen peroxide (1.75×10^{-1} M) was prepared by diluting 1 mL of 30 % H_2O_2 in a 50-mL volumetric flask with deinozed water. Then, the solution was kept in the refrigerator.

Synthesis of ZnO Quantum Dots

ZnO quantum dots were synthesized by following the procedures described by Tang et al. (58). Approximately 44 mg of zinc acetate was dissolved in 20 mL of absolute ethanol. Stirring at room temperature, the mixture was dissolved completely. In addition, 36 mg of LiOH was dissolved in 20 mL of absolute ethanol. The acetate/ethanol solution and the LiOH/ethanol solution were mixed together. The pH value of the mixed solution was measured to be 12. The solution mixture became cloudy, indicating the ZnO nanoparticles were formed after 2 hours of reaction. The pH values of the solutions were tuned to 6, 8, and 10 when 5.5, 10, and 14 mg of LiOH were added, respectively. To remove the un-reacted precursors at the different pH levels,

the synthesized ZnO quantum dots were washed three times with absolute ethanol. Finally, the purified ZnO nanoparticles were dispersed in absolute ethanol in the dark. Quantum dots of different sizes were produced as solutions of different colors were obtained, as reported in the literature (66).

Preparation of Working Solutions

Preparation of Quantum Dots for Obtaining an Emission Spectrum

Exactly 75-μL aliquots of quantum dot solution were diluted in 5-mL volumetric flasks with absolute ethanol. These samples were ready for the fluorescent measurements.

Preparation of Quantum Dot Solutions for Linearity Studies

Exactly 75, 125, 200, 325, and 410 μL solutions of quantum dot solution were diluted in 5-mL volumetric flasks with absolute ethanol. These samples were ready for fluorescent measurements.

Preparation of Quantum Dot Solutions for Immobilization Studies

The fluorescent quantum dot-polymer nanocomposite formation was diluted with 2.0 mL of absolute ethanol. These solutions were ready for fluorescent measurements.

Bis(2,4,6-trichlorophenyl) Oxalate Solution (TCPO)

The TCPO solution (7.5 mg/mL) was prepared by dissolving 0.60 g solid of TCPO in 80 mL of acetonitrile and sonicated for about 1 hour.

Preparation of Hydrogen Peroxide Solutions for Linearity Studies

In different 10-mL volumetric flaks, the calibration solutions were prepared by diluting suitable volumes of 1.0×10^{-1} M H_2O_2. An 8.75×10^{-3} M H_2O_2 solution was prepared by diluting 5.0 mL of 1.75×10^{-1} M H_2O_2 with deinonized water. In addition, 4.38×10^{-3} M, 8.75×10^{-4} M, 4.38×10^{-4} M, 1.75×10^{-4} M, and 8.75×10^{-5} M H_2O_2 solutions were also prepared by diluting, respectively, 2.5 mL, 0.5 mL, 250 µL, 100 µL, and 50 µL of 1.75×10^{-2} M in with deionized water.

Procedure for Immobilization of Quantum Dots

The ZnO quantum dots were immobilized by following the similar procedures described by Yin et al. (67). Approximately 18 mg of ZnO quantum dots were added to 4.0 mL of 1-octadecene to make a quantum dot solution. At the same time, 15 mg of polyisoprene (PI) was dissolved in 40 mL of chloroform to form a polymer solution. Then, the quantum dot solution was added to the polymer solution. In addition, in a separate flask, 15 mg of lauric acid and 4.5 mg of NaOH were dissolved in 40 mL of water to produce an aqueous surfactant solution. The quantum dot/polymer solution was added to the aqueous surfactant solution. ZnO quantum dots were microencapsulated in polyisoprene particles through emulsification/solvent evaporation.

Synthesis of TCPO

TCPO was synthesized by following the procedure described by Mohan and Turro (60). Approximately 25 mL of triethylamine was placed to a 50-mL flask. The triethylamine was distilled. After the distillation, it was cooled to 10^{o}C. At the same time, 300 mL of benzene was dried using 5.0 g of magnesium sulfate. Approximately 20 g of 2,4,6-tricholorophenol was dissolved in 250 mL of the dried benzene and the solution was cooled to 10^{o}C. Then 15 mL of

the distilled triethylamine was added to the mixture. When all was ready, 5.0 mL of oxalyl chloride was added to the mixture drop-wise in a dark room and solid TCPO was produced. The precipitate was filtered and washed three times with benzene and petroleum ether to remove the imparities. The TCPO crystals produced were dried and later stored in amber glass bottle. Produced crystals of TCPO were placed in an amber glass bottle. During synthesis, light was minimized to avoid photo-oxidation of the compounds.

Optimization and Studies

Optimization of Imidazole

Exactly 45, 50, 55, 65, and 70 µL of imidazole solution and 4.0 mL of the TCPO solution were mixed in separate test tubes. Approximately 26 mg of immobilized quantum dots were added to each of the test tubes. Then, exactly 100 µL of 8.75×10^{-3} M H_2O_2 was added to each of the test tubes and the intensity of chemiluminescent produced measured.

Reproducibility Studies

Exactly 55 µL of imidazole solution and 4.0 mL of the TCPO solution were mixed in different test tubes. A single polyisoprene with immobilized quantum dot nanocomposite was added to the test tube. Then, exactly 100 µL of 8.75×10^{-3} M H_2O_2 was added to the each of the test tubes. These samples were ready for chemiluminescent measurements.

Linearity Study Using One Polyisoprene Immobilized Quantum Dot

Exactly 55 µL of imidazole solutions and 4.0 mL of TCPO were mixed in different test tubes. A single polyisoprene immobilized quantum dot was added to the test tube one at a time for measurement. Exactly 100 µL of following H_2O_2 solutions was added to each of different

test tubes after the immobilized quantum dot was added for chemiluminescent measurements: 8.75×10^{-3} M, 4.38×10^{-3} M, 8.75×10^{-4} M, 4.38×10^{-4} M, 1.75×10^{-4} M, and 8.75×10^{-5} M.

Linearity Study Using Multiple Polyisoprene Immobilized Quantum Dot Nanocomposites

Exactly 55 μL of imidazole solution and 4.0 mL of TCPO were mixed in different test tubes. Two polyisoprenes immobilized quantum dot nanocomposites were added to each of the test tube when chemiluminescent measurement was ready to be made. Then, exactly 100 μL of following H_2O_2 was added to each of different test tubes: 8.75×10^{-3} M, 4.38×10^{-3} M, 8.75×10^{-4} M, 5.38×10^{-4} M, and 1.75×10^{-4} M after the addition of the immobilized quantum dot nanocomposites.

Instrumentation

Fluorescence Spectrophotometer

The Perkin-Elmer 650-10s Fluorescence Spectrophotometer was used to measure the intensity of fluorescence from samples. In this instrument, radiation source was a 150 W xenon arc lamp. Samples were placed in a quartz cuvette and the excitation and emission slits were set at 2 nm, and the sensitivity was set at 1. A schematic diagram of the instrumentation is shown in Figure 3.

Chemiluminescence Detector

The instrumentation assembled together to measure the chemiluminescent signals in the laboratory is shown in Figure 4. The Hamamatsu R928 photomultiplier tube (PMT) was used as a detector to provide very low dark currents leading to excellent signal to noise for the low intensity chemiluminescence. In addition, the American Instrument Company microphotomer

(Silver Spring, MD) was used to monitor the signals. These signals were recorded on the Model 680 Hewlett-Packard recorder. In order to achieve the highest levels of sensitivity, the slit was opened to its maximum width and the percent full scale was set on 1. The samples were placed into a transparent glass cuvette suitable for light measurements. The light was measured through a flat surface in order to minimize edge effects. A schematic diagram of the detector is shown in Figure 4.

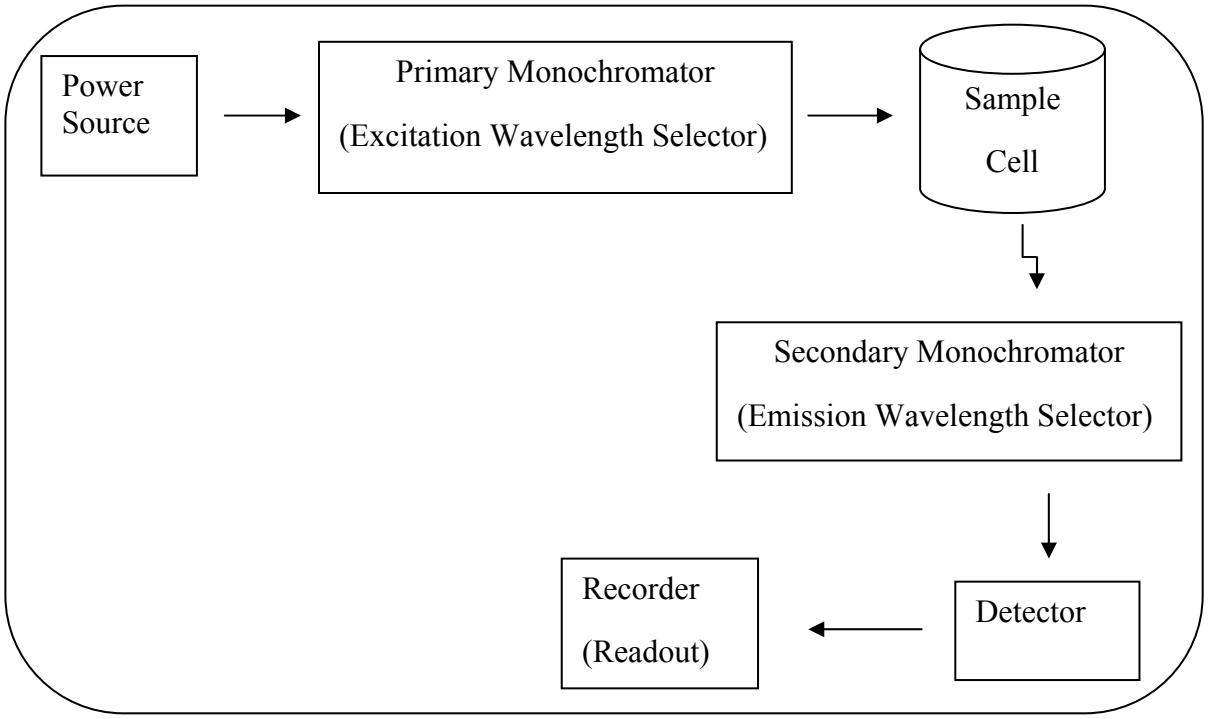

Figure 3. Schematic diagram of the fluorescence spectrophotometer

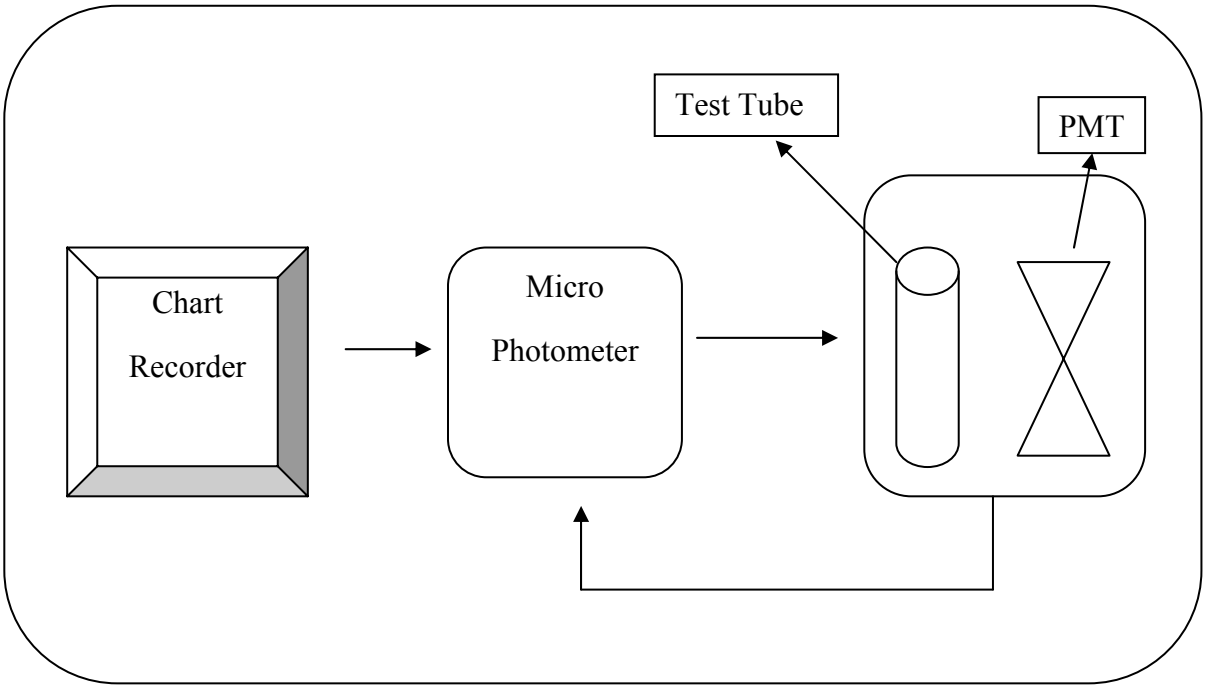

Figure 4. Schematic diagrams for the chemiluminescent detector

CHAPTER 4

RESULTS AND DISCUSSION

In this chapter, the results of various experiments were presented and discussed based upon the intensity of fluorescence and chemiluminescence obtained. The purpose of these experiments was to determine the optimum amount of many reagents used to produce a strong luminescence signal. The results were used to confirm that the synthesis and immobilization of the ZnO quantum dots in a polymeric material is possible and has been done successfully.

Fluorescence Studies of the ZnO Quantum Dot (QD) Solutions

pH-Based QD Synthesis

The suggested method for synthesizing the ZnO quantum dots was a pH-based synthesis repeated previously (66). According to this method, quantum dots of various sizes could be synthesized by controlling the pH of the reacting solution and the QD solution would emit light ranging from the yellow to the red end of the visible spectrum. The size of the quantum dots was controlled by modifying the concentration of LiOH added to control the pH value of the synthetic mixture. At pH 12, ZnO nanoparticles were formed faster and in larger number than those at low pH values. The particles size of the ZnO quantum dots tended to be smaller with an increasing pH value. For example, ZnO nanoparticles prepared at pH 12 exhibited near blue emissions under 309 nm excitations. The emission colors were tuned to green, yellow, and light red by adjusting the pH to 10, 8, or 6, respectively.

Effect of QD Size on the Emission of Light

The ZnO nanoparticles precipitated at various pH values emit different wavelength of light in absolute ethanol under ultraviolet excitation. The color of the quantum dot solutions

visually ranged from yellow to red for those prepared at pH 12, 10, 8, 6, respectively. The results are shown in Table 1.

Table 1. Color of the ZnO QD Solutions Synthesized at Various pH Values and When Exposed to Ultraviolet Light

pH Values	Color (Visually to the Eyes)	Color (Under Ultraviolet Light)
12	Yellow	Blue
10	Orange	Dark Green
8	Dark Orange	Yellow
6	Red	Light Red

The prepared quantum dots were suspended in absolute ethanol, and solutions of various colors were obtained due to differences in their particle size. The larger the quantum dot, the larger the emission wavelength. The emitted wavelength becomes shorter or more towards the blue when the quantum dots shrink in size (36). The real size of the synthesized quantum dots could be measured by using a scanning electron microscope (SEM) or a transmission electron microscope (TEM).

Emission Spectra of QD

The fluorescence spectra were acquired for the solutions of quantum dot synthesized at pH 10. Fluorescence spectra were obtained with the fixed excitation wavelength of 325 nm by scanning the emission wavelengths from 430 to 550 nm. The fluorescence intensity was measured for emission monochromator setting from 430 nm to 550 nm at 10 nm interval. The

maximum emission wavelength occurs at about 495 nm. A plot of such a fluorescence emission spectrum for quantum dots synthesized at pH 10 is shown in Figure 5.

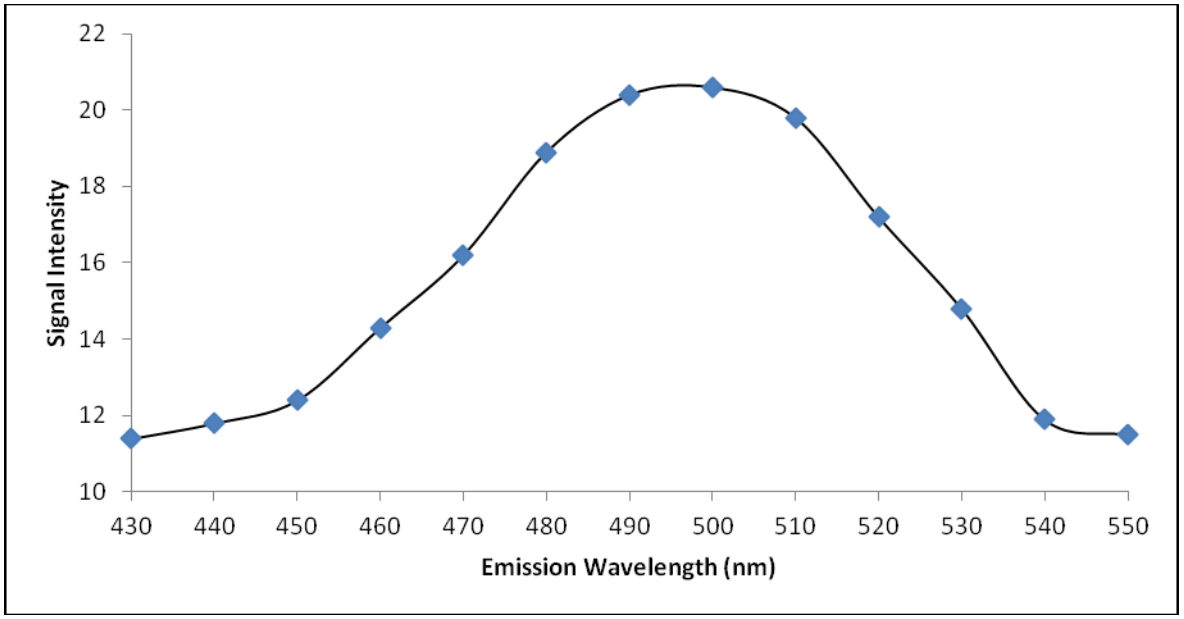

Figure 5. Plot of the fluorescence spectrum of QD synthesized at pH 10 ranging from 430 nm to 550 nm at 10 nm intervals. The excitation wavelength was set at 325 nm. The fluorescence intensity is given in arbitrary units.

This procedure was repeated with all the solutions of the ZnO quantum dots prepared at pH 12, 8, and 6 values. The spectra obtained from these solutions were all similar to that in Figure 5. The wavelengths of maximum fluorescence intensity for the solutions of quantum dots were obtained and the results are shown in Table 2.

Table 2. Maximum Emission Wavelengths for Synthesized Quantum Dots at Different pH Value

pH Values	Maximum Emission Wavelength
12	456
10	495
8	570
6	592

For the larger quantum dots, the maximum fluorescence intensity was found at longer wavelength as predicted. For instance, when the pH value was increased from 6 to 12, the maximum intensity of fluorescence shifted from 592 to 456 nm.

Linearity Studies of Fluorescence Signal with Various Concentration of QD

Various concentrations of ZnO quantum dots solutions were prepared and the fluorescence signals were measured on the Perkin-Elmer 650-10s fluorescence spectrophotometer. Different solutions of the quantum dots synthesized at pH 12 were prepared for fluorescence measurements to see if the intensity is linear with concentrations. The results are shown in Table 3.

Table 3. Measurement of ZnO Quantum Dot Solution Fluorescence Prepared at pH 12 for Linearity Study with an Excitation and Emission Wavelengths 309 nm and 456 nm, Respectively. The Quantum Dots were Dissolved in Absolute Ethanol in 5-mL Volumetric Flasks.

Volume of QD Solutions (μL)	Fluorescence Intensity
0.0	0.0
75	1.9
125	3.1
200	5.5
325	8.7
410	11.2

These results were used to plot a calibration curve. The linear plot is obtained for the QD solution synthesized at pH 12 is shown in Figure 6. From the plot, the fluorescence intensity of the selected quantum dots was shown to be proportional to its concentration. The plot indicated good linearity with a regression coefficient of 0.999. This experiment was repeated with the quantum dots of various sizes synthesized at pH 10, 8, and 6. All the results obtained in the same concentration range were linear.

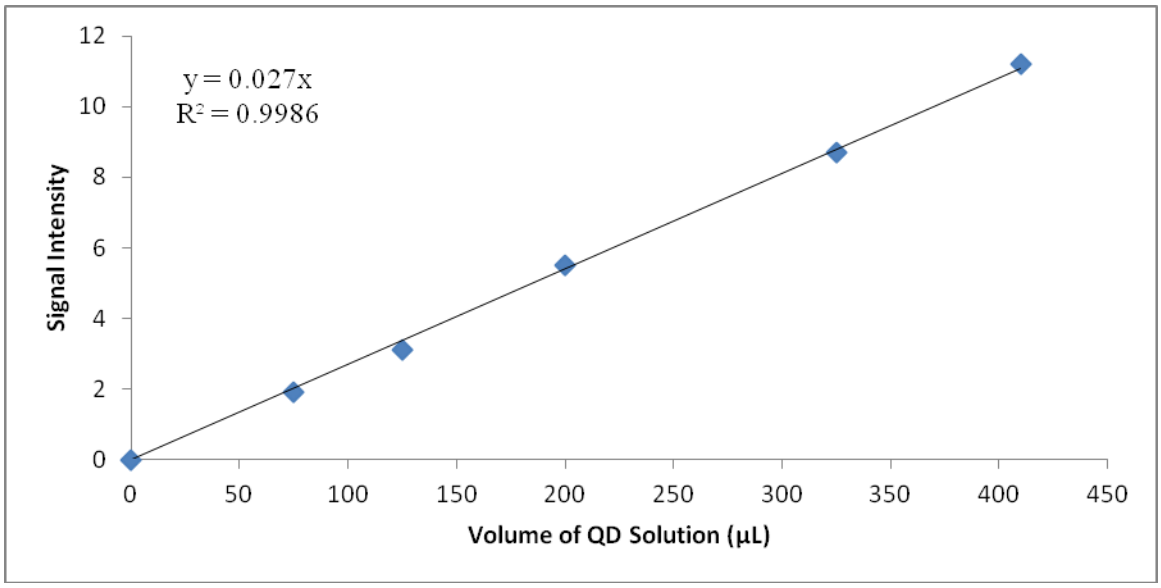

Figure 6. Plot of the fluorescence intensity of ZnO QD solutions synthesized at pH 12 dissolved in absolute ethanol in 5-mL volumetric flasks. The excitation and emission wavelengths were 309 nm and 456 nm, respectively.

Polyisoprene (PI) Immobilization of QDs by Emulsification/Solvent Evaporation Procedure

The surface of QD is highly hydrophobic as synthesized (67). Recently, the encapsulation of quantum dots into polymer colloids has seen growing interest as a route to improve photostability and for development of colorimetric optical bar codes for biological sensing (67). ZnO quantum dots were encapsulated in the core of the polyisoprene (PI) particles during emulsification/solvent evaporation (67). The PI was chosen because it is soluble in many of the same solvents as the QDs, nontoxic, and is biocompatible (67). For these studies, the immobilized quantum dots prepared at pH 6 were tried.

The quantum dot solutions to have the QD particle to be incorporated into the PI polymer were stirred for 2 hours and the solution decanted from the mixture. The decanted solutions were stored in the dark and their fluorescence measured later. The spectrum of the immobilized QD synthesized at pH 6 shown in Figure 7.

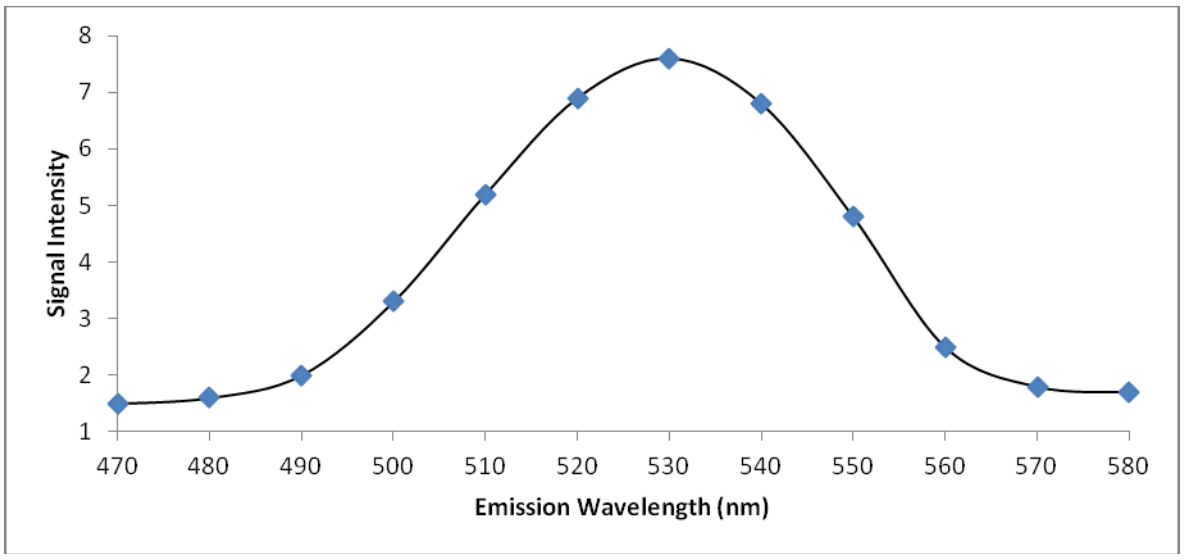

Figure 7. Plot of the fluorescence spectrum of PI polymer immobilized QD synthesized at pH 6. The excitation wavelength was set at 356 nm, and the emission wavelength ranged from 470 nm to 580 nm.

After carefully observing the immobilized product, it seems that the quantum dots were immobilized into PI only to a small extent. The PI was easily cross-linked during the encapsulation process to enhance water stability of the QD (67). The PI QD/nanocomposite formed a stable suspension in water that exhibits stable fluorescence for months. A small fraction of the quantum dots in the solution was immobilized on the PI possibly due to polarity differences. The PI immobilized QD/nanocomposite did not change significantly the fluorescence intensity compared to the non-immobilized ZnO QD solutions.

More QDs bind to the surface of the PI with higher concentration of PI. This led to higher luminescence intensity as shown in Table 4. In addition, as the concentration of PI dissolved in chloroform was increased, both the particle size and size polydispersity increased due to the increase in solution viscosity (67). Therefore, the size of quantum dot can be modified by adjusting the concentration of PI.

Table 4. Measurements of the PI Immobilized ZnO QDs Fluorescence Intensities at pH 6 with Varying PI Concentrations. Excitation and Emission Wavelengths Were 356 nm and 590 nm, Respectively.

Signal Intensity (0.28 g of PI)	Signal Intensity (0.15 g of PI)
0.068	0.044
0.070	0.052
0.074	0.048
0.075	0.050

Another procedure to immobilize the QD was also tried. This method is a slight modification of the procedure used before. The ZnO QD/polymer nanocomposite was prepared by using only dissolved PI in chloroform without the addition of lauric acid and NaOH. The fluorescence intensity of the immobilized QD was lower than that obtained by previous method. The fluorescent spectrum obtained from the immobilized QD was narrower and not as broad as shown in Figure 8. It seems that by submerging the quantum dot solution in PI aggregation and size distribution of QD modified when they were adsorbed onto the only PI. Another observation is that not only is the fluorescence spectrum not as broad, the peak maximum is also shifted slightly from 590 nm to 485 nm compared to the non-immobilized QD solution.

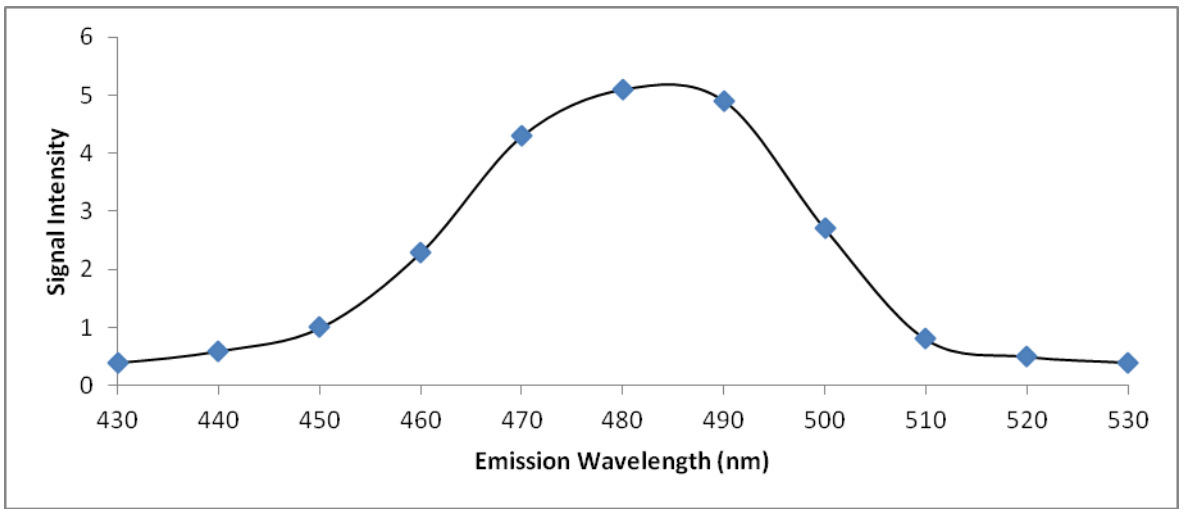

Figure 8. Plot of the fluorescence spectrum of immobilized QD nanocomposite is in only PI polymer at pH 6. The excitation wavelength was set 356 nm, and the emission wavelength ranged from 430 nm to 530 nm.

TCPO-CL Studies of PI Immobilized QD

Peroxyoxalate chemiluminescence (CL) takes place when an electronically excited species transfers energy to a fluorophore that luminesces. In the presence of an imidazole catalyst, the TCPO reacts with hydrogen peroxide faster to yield more intense chemiluminescence.

Chemiluminescence has become widely applied in analysis for several reasons. It is extremely sensitive and selective. In addition, the simplicity of the instrumentation is a major advantage as chemiluminescence techniques require only a photomultiplier tube (PMT) and a reaction vessel. The excitation energy comes from the reaction between the analyte and the reagent, thus no radiation source is required and this also avoids the problem with scattered or stray radiation. Because the detection limits are dependent upon reagent purity, the detection limits lie between the part-per-billion and the part-per-million range (59).

Quantum dots were immobilized in polyisoprenes (PI) polymer by the first method (67). The immobilized quantum dots were studied to validate their feasibility as a reusable and useful fluorophore in TCPO-CL analysis. Immobilized PI- QDs were obtained using the pH 12 synthesized quantum dot solutions. The PI encapsulated quantum dots were used in TCPO-CL studies: optimization of imidazole, reproducibility studies, and linearity studies.

Optimization of Concentration of Imidazole Catalyst Solution

The reaction between TCPO and hydrogen peroxide can be catalyzed by bases such as imidazole. A flow injection system was used to measure CL from the peroxyoxalate reaction. The optimum concentration of imidazole used to obtain the highest CL signal was studied. Approximately 26 mg of the immobilized quantum dot/nanocomposite and 4.0 mL of TCPO solution (7.5 mg/mL), and different concentrations of imidazole were placed in different test tubes. And then, exactly 100 μL of 8.75 x 10^{-3} M H_2O_2 was added to each of the test tubes. The instrumentation assembled in the laboratory was used to determine the CL intensities, which are tabulated in Table 5. These results are also plotted in Figure 9.

Table 5. Results of the Optimization of Imidazole for TCPO-Hydrogen Peroxide CL Reactions

Volume of Stock Imidazole Added (μL)	CL Signal Intensity
45	0.013
50	0.028
55	0.048
65	0.024
70	0.018

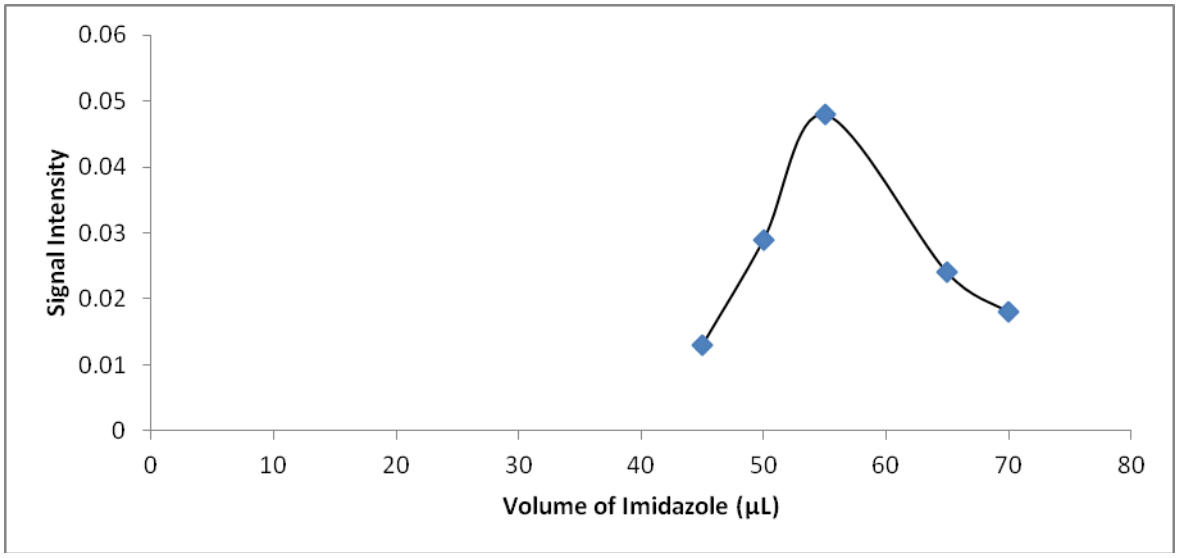

Figure 9. Plot of the results of the optimization of imidazole concentration for the TCPO (7.5 mg/mL)-hydrogen peroxide (8.75 x 10^{-3} M) reaction. The concentration of the stock imidazole solution was 100 mg/mL. The CL intensity is giving in arbitrary units.

CL intensity was found to continue to increase until it reaches a maximum and then decreasing after that. The highest CL intensity was obtained when 55 µL of the stock imidazole solution of concentration 100 mg/mL was added to the 5-mL volumetric flask. The CL intensity decreased when the volume of imidazole used was greater than 55 µL. According to these results, 55 µL of the 100 mg/mL stock imidazole solution was used in all of the subsequent TCPO-CL reactions.

Optimization of the Concentration of TCPO

The optimum amount of the TCPO to produce the highest possible signal with a given flow rate was determined in this experiment. The amount of imidazole used was 55 µL (1.75 mg/mL) and 8.75 x 10^{-3} M H_2O_2 was kept constant while the concentration of TCPO used was

varied. The results of the experiment are shown in Table 6 and a plot of the data acquired shown in Figure 10.

Table 6. Results of Optimization of TCPO for PI Polymer Immobilized QDs CL Reaction at pH 12

Amount of TCPO (mg)	CL Signal Intensity
100	0.022
200	0.037
300	0.043
400	0.048
500	0.050
600	0.050

The results showed that CL intensity increased with an increase in the TCPO concentration. The CL intensity reached a plateau and did not increase much beyond the TCPO concentration of 7.5 mg/mL. From this experiment, it was determined that 7.5 mg/mL of TCPO solution was the optimum concentration of TCPO was adequate.

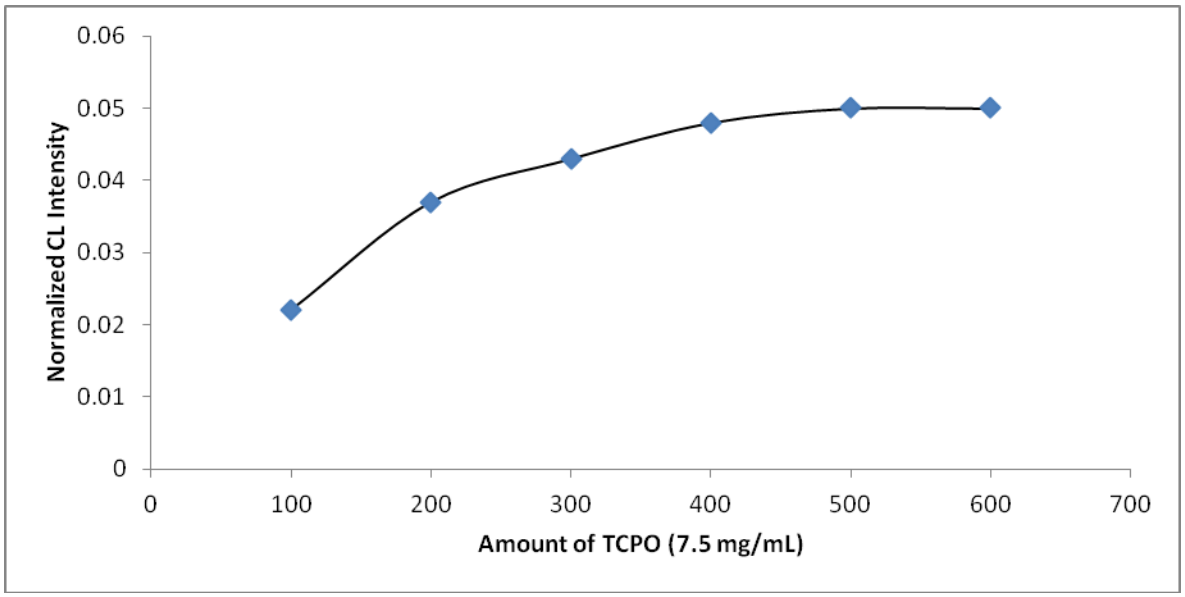

Figure 10. Plot of the results of the optimization of TCPO experiment for the TCPO (7.5 mg/mL)-hydrogen peroxide (8.75 x 10^{-3} M) reaction. The concentration of stock TCPO solution was 100 mg/mL. The CL intensity is given in arbitrary units.

Reproducibility Studies

To be useful for routine analysis, an analytical method must be reproducible with high precision. Two different experiments were conducted to establish the reproducibility of chemiluminescence method. In the first experiment, five different 8.75 x 10^{-3} M H_2O_2 solutions were prepared in separate 10-mL volumetric flasks. The CL intensity was measured from the PI immobilized QDs by reacting 4.0 mL of TCPO and 55 μL of stock imidazole with the varying hydrogen peroxide solutions. In this experiment, the same single PI immobilized quantum dots were used. Triplicate CL measurements of each hydrogen peroxide concentration were acquired, and then the mean and RSD for each solution were calculated. The results of the experiment are shown in Table 7.

Table 7. Results of reproducibility study on five different 8.75 x 10^{-4} M hydrogen peroxide solutions under identical condition of imidazole and TCPO

Trial	1	2	3	4	5
CL Intensity 1	0.0050	0.0052	0.0048	0.0047	0.0048
CL Intensity 2	0.0048	0.0048	0.0046	0.0048	0.0051
CL Intensity 3	0.0047	0.0047	0.0048	0.0051	0.0048
Mean	0.0048	0.0049	0.0048	0.0049	0.0049
RSD (%)	3.16	5.39	0.0047	4.27	3.53
Total Mean	0.0048				
RSD (%)	3.75				

Precision of the data obtained for the five different hydrogen peroxide solutions are all less than an RSD of 10 %. The mean of the total 15 CL measurements was 0.0048, with an RSD of 3.75 %. The outcomes showed that the precision of the analytical method was acceptable. The CL intensity from the different hydrogen peroxide concentration solutions was precise with the RSD value of less than 10 %. In addition, it exhibited that the PI immobilized quantum dots could be used many times without a substantial change in CL intensity.

The second part of experiment used only one 8.75 x 10^{-4} M hydrogen peroxide solution with CL of one immobilized QD measured over a period of time. Exactly 4.0 mL of TCPO and 55 µL of imidazole reacted with 100 µL of the hydrogen peroxide solution. This investigation was carried out many times over several weeks using the same single PI immobilized quantum dots. For this experiment, hydrogen peroxide solutions of the equal concentrations were freshly

prepared. The experiment was performed to check the stability of the PI immobilized quantum dots. The results of this experiment are shown in Table 8.

Table 8. Results of Reproducibility Study of Using a Single PI immobilized QD. Fixed volumes of TCPO (7.5 mg/mL) and Imidazole (100 mg/mL) reacted with 1.0 mL of 8.75×10^{-4} M Hydrogen Peroxide Solution.

Trial	1	2	3	4	5	6
CL	0.0047	0.0045	0.0040	0.0036	0.0035	0.0033
Trial	7	8	9	10	11	12
CL	0.0031	0.0028	0.0021	0.0020	0.0017	0.00014
Mean	0.0031					
RSD (%)	12.37					

Based on the data obtained, the mean was 0.0031, with an RSD of 12.37 % over. It was concluded that the same immobilized quantum dots could be used for analysis at least 29 times over 9 weeks and still gave good results. This demonstrated that the PI immobilized QD remained stable and persisted to luminesce over several months at a time. The CL intensity decreased slightly as more analysis was done. Eventually after 29 uses and 9 weeks, the CL intensity became weak and undetectable. These results are plotted in Figure 11.

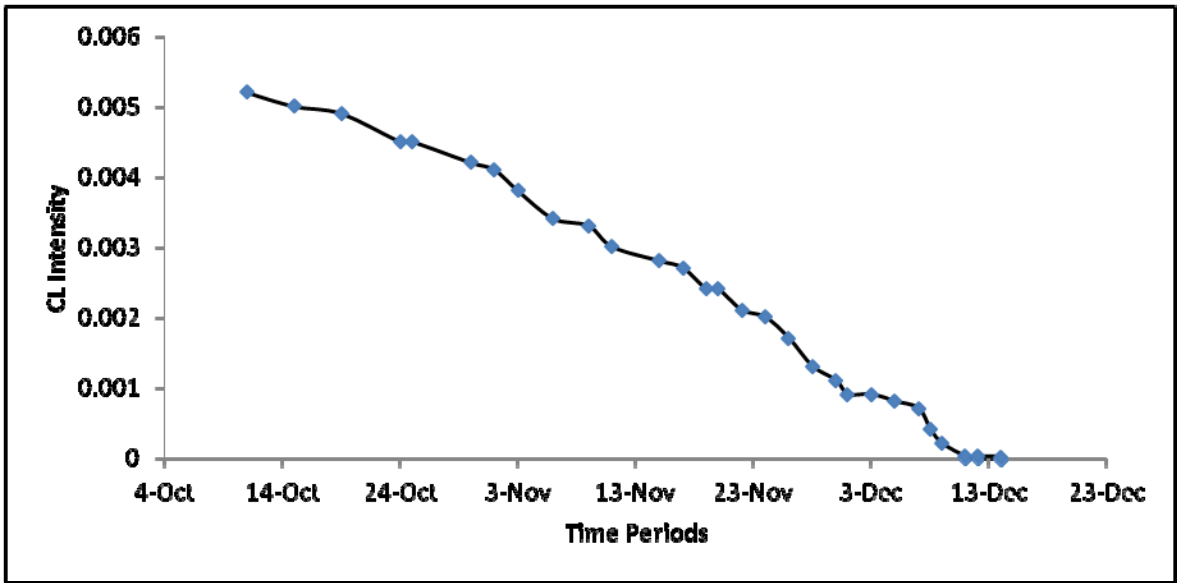

Figure 11. Plot of the results of the CL PI immobilized QD at pH 12 within several months. TCPO (7.5 mg/mL)-hydrogen peroxide (8.75×10^{-3} M) reaction. The concentration of stock TCPO solution was 100 mg/mL.

Linearity Studies of CL Intensity with H_2O_2 Concentration Using PI Immobilized QD

One PI Immobilized QD. Linearity studies were performed by reacting TCPO with different concentrations of hydrogen peroxide to yield CL using a single PI immobilized quantum dots prepared previously. Triplicate hydrogen peroxide solutions of a given concentrations were prepared individually. For triplicate solutions, duplicate CL values were acquired. Approximately 0.26 g of one piece of immobilized quantum dot/polymer nanocomposite was used in this particular experiment. The results of the experiment are shown in Table 9.

Table 9. Results of linearity studies of CL intensity with hydrogen peroxide using a single PI immobilized QD

H_2O_2 (M)	8.75×10^{-3}	4.38×10^{-3}	8.75×10^{-4}	4.38×10^{-4}	1.75×10^{-4}
CL Intensity 1	0.0520	0.0240	0.0050	0.0024	0.00096
	0.0480	0.0230	0.0048	0.0023	0.00070
CL Intensity 2	0.0470	0.0240	0.0047	0.0022	0.00080
	0.0480	0.0230	0.0052	0.0022	0.00086
CL Intensity 3	0.0500	0.0240	0.0048	0.0023	0.00070
	0.0450	0.0230	0.0047	0.0024	0.00065
Mean	0.04835	0.0235	0.00485	0.0023	0.00077
RSD (%)	5.34	2.45	4.28	4.35	16.19

The RSD (%) and mean values were determined because the CL intensity was measured multiple times. The hydrogen peroxide concentrations with the corresponding the mean CL intensities are plotted in Figure 12.

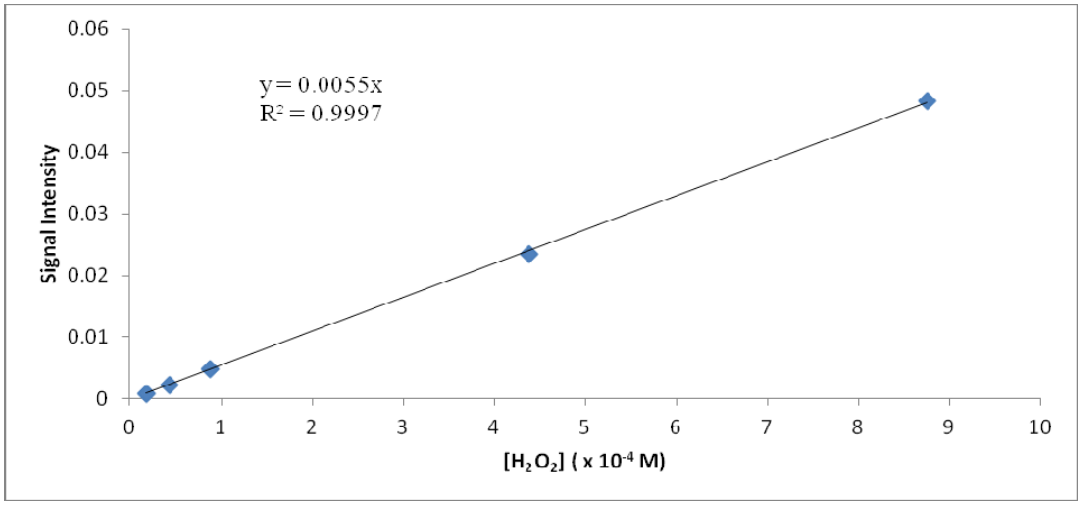

Figure 12. Plot for mean duplicate CL measurements on each individually prepared triplicate hydrogen peroxide solution using 4.0 mL of TCPO, 55 µL of imidazole, and a single PI immobilized QD.

At lower concentrations of hydrogen peroxide, more fluctuations were observed in the CL intensities. It is demonstrated with the RSD values obtained. The RSD value for the lowest concentration of hydrogen peroxide, 1.75 x 10^{-4} M, was 16.19 % while at the same time all the other concentration of H_2O_2 was less than 5.36 %. The useful linearity limit was from 8.75 x 10^{-4} M to 4.38 x 10^{-4} M H_2O_2 solution. The linearity curve is displayed in Figure 13.

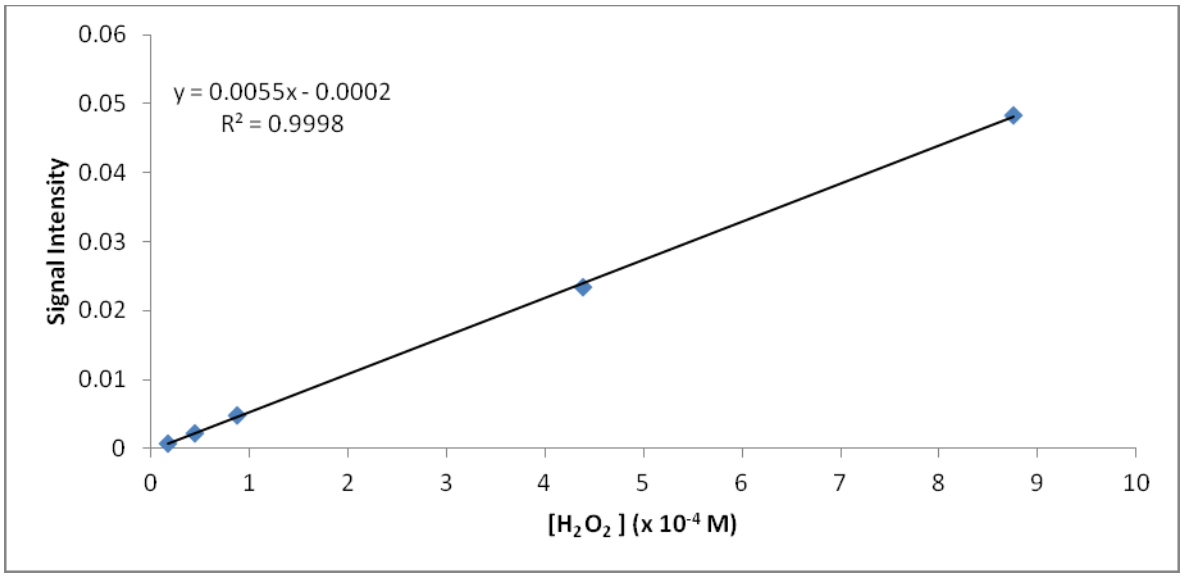

Figure 13. Plot of linearity curve of the average CL measurements on each separately synthesized hydrogen peroxide solution using 4.0 mL of TCPO, 55 µL of imidazole, and a single PI immobilized QD.

Multiple PI Immobilized QD Pieces. The goal of this experiment was to investigate if a better CL intensity could be measured with multiple PI immobilized quantum dot pieces used together in CL measurement. CL intensity was measured for different concentration of H_2O_2 added to a fixed amount of TCPO and imidazole mixture. Approximately 0.42 g of multiple polyisoprene immobilized quantum dot pieces instead of one single large piece were used. The CL values for each hydrogen peroxide solution were acquired in triplicates. The outcomes of the

experiments are shown in Table 10. The hydrogen peroxide concentrations with equivalent mean CL values are plotted in Figure 14.

Table 10. Results of linearity studies of CL intensity with various concentrations of hydrogen peroxide using multiple PI immobilized QD

H_2O_2 (M)	8.75×10^{-3}	4.38×10^{-3}	8.75×10^{-4}	4.38×10^{-4}	1.75×10^{-4}
CL Intensity 1	0.0640	0.0290	0.0057	0.0027	0.00098
CL Intensity 2	0.0630	0.0270	0.0058	0.0025	0.00086
CL Intensity 3	0.0600	0.0280	0.0053	0.0024	0.00084
Mean	0.0623	0.0280	0.0055	0.0025	0.00089
RSD (%)	3.33	3.57	4.81	6.03	8.47

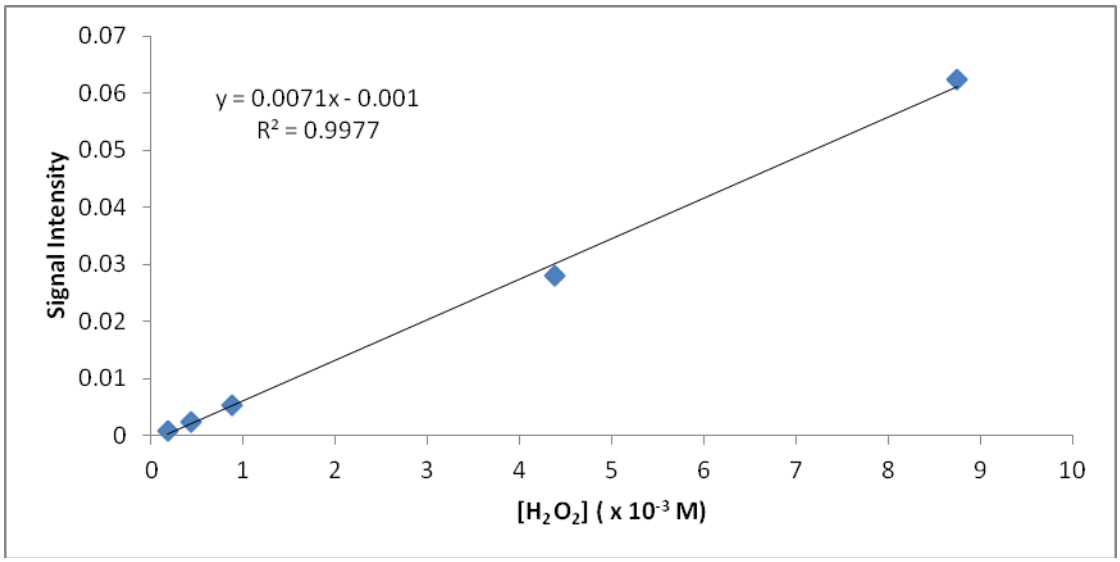

Figure 14. Plot of the mean triplicate CL measurements on each hydrogen peroxide solution using 4.0 mL of TCPO, 55 µL of imidazole, and multiple PI immobilized QD

In general, the CL intensities of the multiple polyisoprenes immobilized quantum dots pieces were linear similar to the results obtained when a single piece of polyisoprene

immobilized quantum dots was used. However, the CL intensity at the same hydrogen peroxide concentration seems to be somewhat lower even though more pieces were used. In addition, the lowest concentration of hydrogen peroxide solution gave higher RSD for the CL intensities measured. The workable dynamic linear range was from 4.38×10^{-4} M to 1.75×10^{-4} M, as shown in Figure 15. The using multiple PI immobilized QD does not increase the CL intensity much and may actually hurts the results. The outcomes of the experiments are shown in Table 11.

Table 11. Results of linearity studies of CL intensity with multiple PI immobilized QD pieces

H_2O_2 (M)	8.75×10^{-3}	4.38×10^{-3}	8.75×10^{-4}	4.38×10^{-4}	1.75×10^{-4}
CL Intensity 1	0.0650	0.0310	0.0058	0.0025	0.00098
CL Intensity 2	0.0590	0.0280	0.0057	0.0027	0.00078
CL Intensity 3	0.0630	0.0270	0.0052	0.0031	0.00081
Mean	0.0623	0.0287	0.0056	0.0028	0.00086
RSD (%)	4.9012	7.2616	5.7746	11.0424	12.59042

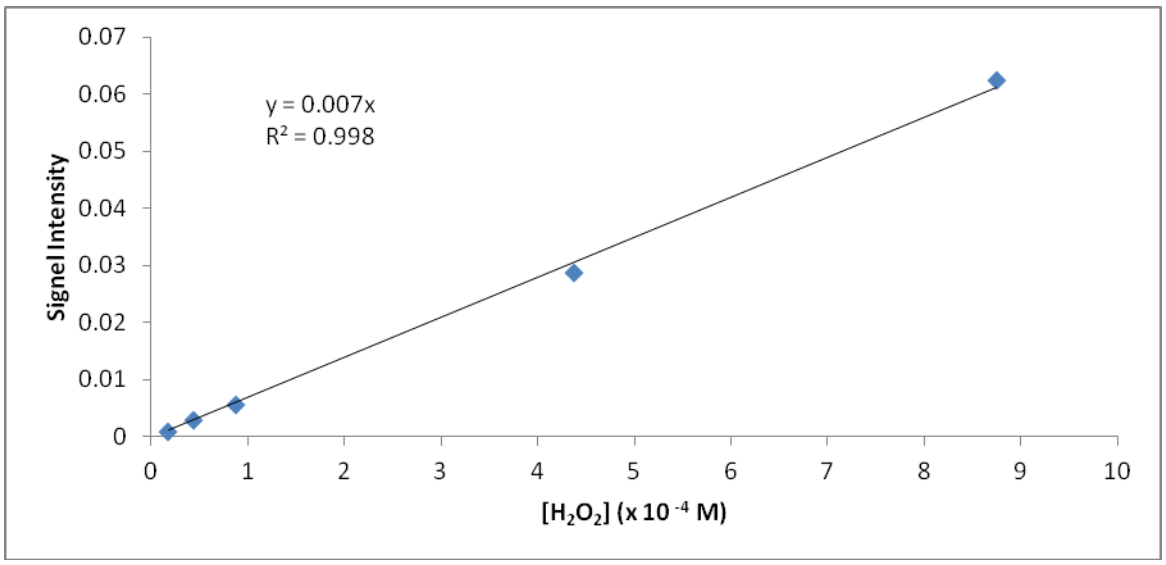

Figure 15. Plot of the linearity curve of the mean CL values on the each hydrogen peroxide solution using 4.0 mL of TCPO, 55µL of imidazole, and multiple PI immobilized QD. The linearity range is from 4.38 x 10^{-3} M to 1.75 x 10^{-4} M hydrogen peroxide solution.

CHAPTER 5

CONCLUSION

The synthesis of ZnO quantum dots has been carried out and their luminescence properties studied successfully. The usefulness of the polyisoprene immobilized ZnO quantum dots as a reusable was established and multiple uses were accomplished.

The proposed process for synthesizing ZnO quantum dots proved to be adequate. The pH-based synthesis of quantum dots was successfully and the emitted QD light ranging from the blue to red. The smaller quantum dots emitted shorter wavelength of light, while the larger quantum dots emitted longer wavelengths. The size of the quantum dots was controlled by pH-adjustment of the LiOH solutions. Good yield was obtained.

The fluorescence properties of quantum dots were examined with various experiments. The maximum fluorescence intensity was achieved at longer emission wavelengths for larger quantum dots and vice-verse for the smaller quantum dots as predicted. The fluorescence intensity of the quantum dot solution was found to be linear with the concentration of the QD solutions.

The immobilization of quantum dots into biocompatible polyisoprene (PI) particle using an emulsification/solvent evaporation method was successful. Adequate amount of the ZnO quantum dots were encapsulated into the PI surface because the surface chemistry of both is alike. Emusification/solvent evaporation results in the quantum dots immobilized into the polyisoprene particle core without chemical modification of the quantum dots. The polyisoprene/quantum dot nanocomposite is stable and exhibited stable fluorescence for about three months.

The PI encapsulated QD provided reproducible CL data with satisfactory precision and linear dynamic range. It can luminesce over several weeks at a time when kept in a dark room. After the immobilized quantum dot was used multiple times and for extended periods of time, its capability to luminesce start to decrease only slowly. Thus the PI immobilized QD proved to be a reusable and stable fluorophore.

It was predicted that the chemiluminescence intensity would increase as the number of individual pieces of the PI immobilized quantum dots used in CL was in use and exposed to the TCPO-hydrogen peroxide were better. The data acquired however showed that the CL intensity obtained a single piece of PI immobilized quantum dots was used.

The exact size of the quantum dots could be measured using a scanning electron microscope (SEM) or a transmission electron microscopy (TEM). In addition, more research regarding the encapsulation on surfaces of various particles could be conducted. The PI immobilized QDs used for CL measurements.

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